FLUORESCENCE IMMUNOASSAY USING WATER INSOLUBLE DYES

FIELD OF THE INVENTION

This invention relates to an immunoassay of an analyte and materials used therein, and more particularly relates to a method and materials for time resolved immunoassay in which a label is encapsulated in a vesicle.

BACKGROUND OF THE INVENTION

A variety of assay systems which are both rapid and sensitive has been developed to determine the concentration of a substance in a fluid. Immunoassays depend on the binding of an antigen or hapten to a specific antibody and have been particularly useful because they give high levels of specificity and sensitivity. These assays generally employ one of the above reagents in labeled form, the labeled reagent often being referred to as the tracer, and may be carried out in solution or on a solid support.

Radioimmunoassay (RIA) procedures use radioisotopes as labels, provide high levels of sensitivity and reproducibility, and are amenable to automation for rapid processing of large number of samples. However, isotopes are costly, have relatively short shelf lives, require expensive and complex equipment, and extensive safety measures for their handling and disposal must be followed.

Enzymes have also been used as labels in immunoassay. Enzyme immunoassay (EIA) wherein an enzyme label is encapsulated in a vesicle later lysed by complement is disclosed in U.S. Pat. No. 4,235,792 to Hsia et al. and U.S. Pat. No. 4,342,826 to Cole. EIA, although improving on RIA because it does not require precautions against radioactivity, nevertheless has disadvantages. EIA depends on the reaction of the enzyme with a substrate to produce a color which is measured, and thus requires the additional step of providing an enzyme substrate. In addition, sufficient time must be allowed for color development, and an expensive spectrophotometer for measuring color change often must be provided.

Fluoroimmunoassay (FIA), in contrast to EIA, provides direct detection of the label. FIA procedures in which the dyes are entrapped or embedded in either the aqueous phase or the lipid phase of a liposome are disclosed in U.S. Pat. No. 4,372,745 to Mandle et al. and International Published Application No. WO 85/00664 to Kunq et al. In Mandle et al., the liposome is disrupted after a binding reaction and the dye is excited by chemiluminescence generated in the assay medium. Kung et al. is an agglutination assay and requires a large liposome for dye-aided visual detection of an agglutinate.

Known FIA methods using organic fluorochromes, such as fluorescein or rhodamine derivatives, have not achieved the high sensitivity of RIA or EIA, largely because of light scattering by impurities suspended in the assay medium and by background fluorescence emission from other fluorescent materials present in the assay medium. Scattering is particularly troublesome with fluorochromes having a short (50 nm or less) Stoke's shift (the difference between the wavelength of the absorption and emission). For example, the Stoke's shift of fluorescein isothiocyanate is only 20–30 nm. Background fluorescence is particularly troublesome when the assay medium is serum. The sensitivity of an assay in serum may be reduced up to one hundred fold compared to an identical assay in buffer.

The development of time-resolved fluoroimmunoassay (TR-FIA) has contributed to overcoming these problems In this procedure, a fluorochrome label with relatively long fluorescence emission decay time is excited with a pulse of light, and fluorescence emission from the label is measured after a preselected delay. Background emission of short decay time (generally less than 10 ns) essentially ceases during the delay and thereby does not interfere with measurement of the specific emission from the label. TR-FIA is most effective when the fluorescent label has a decay time of 100–1000 ns and a long Stoke's shift (100 nm or greater).

A class of labels meeting the requirements of TR-FIA is the lanthanide chelates. Lanthanide ions, such as ions of europium and terbium, though not fluorescent themselves, form highly fluorescent chelates of long Stoke's shift (up to 250 nm) with organic ligands, in particular with β-diketones. The ligand portion of the chelate absorbs excitation light and transfers the absorbed energy to the chelated metal ion. The metal ion emits the energy as fluorescence of exceptionally long decay time (1 ms). A discussion of the use of lanthanide chelates in TR-FIA is given in Analytical Biochemistry, 137, 335 (1984) and in Clinical Biochemical Analysis 14, 71 (1984).

U.S. Pat. No. 4,058,732 to Wieder discloses a method and apparatus for use of lanthanide chelates and time resolution in analytical fluorescent spectroscopy.

U.S. Pat. No. 4,283,382 to Frank et al. discloses an improvement in TR-FIA in which a lanthanide chelate label is incorporated into a polymeric bead lattice to eliminate water-induced quenching of the fluorescence emission of the label.

U.S. Pat. No. 4,374,120 to Soini et al. discloses increased stability of lanthanide chelates achieved by a 1:1:1 chelate of lanthanide, β-diketone, and an aminopolycarboxylic acid analogue having a functional group useful for binding the chelate to a protein.

European Pat. Application EP 0,064,484-A2 discloses a TR-FIA procedure in which the substance to be determined is coupled to a lanthanide by an aminocarboxylic acid analogue, and, after incubation, the lanthanide is split from the substance to be determined and chelated to a β-diketone before detection.

Copending application Ser. No. 049,971, filed May 15, 1987, of common assignee, discloses a rare earth ion chelate encapsulated in a sac, which ion is fluorescent when released from the sac and complexed with an activator. The sac may be conjugated to a ligand and used in a TR-FIA.

Truneh et al. (Journal of Immunological Methods, 100 59 (1987)) describes enhancement of the fluorescent signal in cell labeling experiments by encapsulating water soluble fluorescein and rhodamine dyes in the aqueous compartment of liposomes, and suggests that intercalating hydrophobic dyes in the liposomal membrane may enable such dyes to be used in similar fashion.

Although lanthanide chelate labels are useful in FIA, several problems exist. Covalent attachment of the chelating agent to a protein component of the assay is a time consuming operation requiring complex chemical reactions under carefully controlled conditions. Such covalent bonding of the chelating agent to the protein may additionally reduce the stability of the subse-